COL10 expression in the limb cartilage. Under the OA induction in the wild-type joints, C/EBPβ was induced at the frontline of cartilage destruction, whereas in the C/EBPβ+/– joints, the destruction as well as chondrocyte hypertrophy and COL10 expression were significantly suppressed. In the ex vivo culture of C/EBPβ–/– costal chondrocytes, COL10 expression was significantly decreased compared to the wild-type culture. The mRNA level and promoter activity of COL10 were enhanced by the C/EBPβ transfection, and the core responsive region of the COL10 promoter was identified between -81 and -76 bp relative to the transcriptional start site. Since C/EBPβ and Runx2 are known to function as mutual transcriptional co-factors, we further generated C/EBPβ and Runx2 compound deficient (C/EBPβ–/–; Runx2+/–) mice. The C/EBPβ–/–; Runx2+/– mice exhibited severer dwarfism than the C/EBPβ–/– mice. Although chondrocyte hypertrophy and COL10 expression were comparable between the two genotypes, cartilage degradation and MMP13 expression were markedly suppressed by the Runx2 insufficiency. In the culture of SW1353 cells, co-transfection of C/EBPβ and Runx2 enhanced MMP13 expression, but not proliferation or COL10 expression, as compared to a single transfection of C/EBPβ or Runx2. The promoter activity of MMP13 was synergistically enhanced by the co-transfection, and the core responsive region was identified between -111 and -89 bp, which contains a C/EBP-binding motif, but not a Runx2-binding motif.

Conclusions: C/EBPβ is a crucial transcription factor for chondrocyte hypertrophy and cartilage degradation. Runx2 contributes to the latter step as the co-factor, but not to the former step, indicating distinct transcriptional control of these sequential steps during endochondral ossification by C/EBPβ and Runx2.

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MiR-140 IS EXPRESSED IN DIFFERENTIATED HUMAN ARTICULAR CHONDROCYTES AND MODULATES IL-1 RESPONSES

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Purpose: MicroRNAs (miRNAs) are a class of noncoding small RNAs that act as negative regulators of gene expression. The miRNAs exhibit tissue-specific expression patterns and changes in their expression may contribute to pathogenesis. The objectives of this study were to identify miRNAs expressed in articular chondrocytes, determine changes in osteoarthritic cartilage and address the function of miR-140.

Methods: To identify miRNAs specifically expressed in chondrocytes, we performed gene expression profiling using miRNA microarrays and quantitative PCR with human articular chondrocytes compared to human mesenchymal stem cells (MSC). The expression pattern of miR-140 was monitored during chondrogenic differentiation of hMSC in pellet cultures and in human articular cartilage from normal and osteoarthritic knee joints. We tested effects of IL-1β on miR-140 expression. Double-strand (ds) miR-140 was transfected into chondrocytes to analyze changes in the expression of genes associated with osteoarthritis.

Results: Microarray analysis showed that miR-140 has the largest difference in expression between chondrocytes and MSC. During chondrogenesis cultures of MSC miR-140 expression increased in parallel with Sox9 and Col2a1. Normal human articular cartilage expressed miR-140 and this was significantly reduced in OA tissue. In vitro treatment of chondrocytes with IL-1β suppressed miR-140 expression.In contrast to miR-140, miR-146 has a broader tissue distribution, it is increased in response to IL-1, it is upregulated in OA. Transfection of chondrocytes with ds-miR-140 downregulated IL-1β-induced ADAMTS-5 expression and rescued the IL-1β -dependent repression of Aggrecan gene expression.Moreover, we performed searches in three databases (“TargetScan”, “ PicTar”, “miRbase”) and this yielded 223-975 potential miR-140 targets. Only 9 potential targets were identified in all three databases.

Conclusions: This study shows that miR-140 has a chondrocyte differentiation-related expression pattern. The reduction in miR-140 expression in OA cartilage and in response to IL-1β may contribute to the abnormal gene expression pattern characteristic of OA.

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THE ADIPOKINE, RESISTIN, INDUCES A HIGH LEVEL OF EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: To provide a picture of the effect of resistin on human articular chondrocyte gene expression of cytokines and chemokines.

Methods: Chondrocytes were obtained from conserved area of cartilage from donors undergoing total knee joint replacement surgery. Chondrocytes were isolated and plated at a density of 2.5 ×105 cells/cm^2 in Dulbecco’s modified eagle’s medium (DMEM)/F12 media plus 10% fetal bovine serum (FBS), 50 mg/ml ascorbate and antibiotics (50 U/ml penicillin and 50 mg/ml streptomycin) for 24 h. Serum was removed and cells were allowed to recover for 24 h before adding resistin from BioVision (Mountain view, CA) for 24 h. Changes in gene expression were analyzed by quantitative real-time polymerase chain reaction.

Results: Resistin treated human articular chondrocytes showed significant increases in the expression of a large group of cytokines and chemokines, including IL-1a, IL-1b, IL-6, IL-8, CCL3, CL4, CCL8, CXCL1, CXCL3, CXCL6. As expected, the mRNA for matrix metalloproteinase (MMP)-1, MMP-3 and BMP2 also increased, but not as much as the above genes. Genes were placed in three